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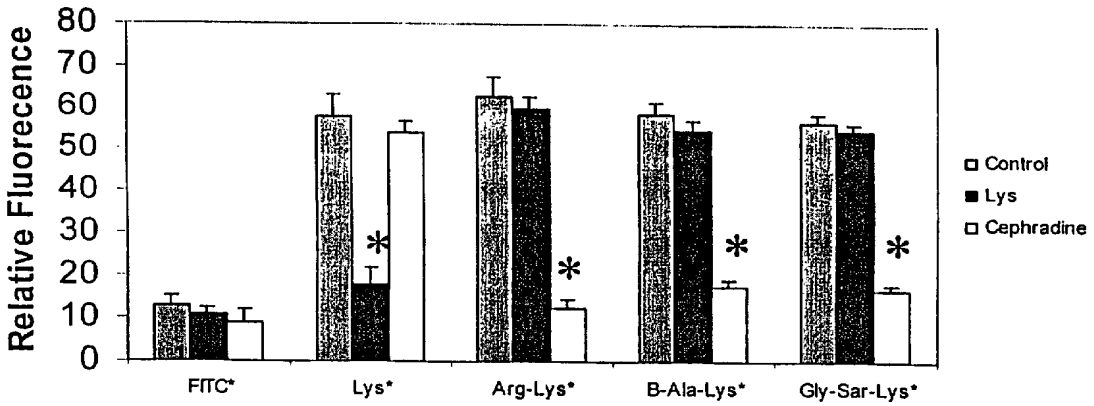

*Significantly lower than control. $P < 0.05$ $N = 6$

(57) Abstract: A composition comprising one or more nitric oxide donors and an antioxidant is disclosed. The nitric oxide donor of the present invention provide a substrate for the generation of nitric oxide; coupled with the antioxidant, the present compositions therefore serve to raise nitric oxide levels in a patient while minimizing peroxynitrite production. As a result, vasorelaxation or vasodilation is experienced without the tissue and cellular damage associated with other methods of NO generation. Methods for treating a patient for illnesses in which vasoconstriction occurs or is a symptom are therefore also disclosed. The methods generally comprise administering a NO donor concurrently or sequentially with an antioxidant to the patient to be treated in an amount effective to induce the desired level of vasorelaxation.

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COMPOSITIONS AND METHODS FOR INDUCING VASORELAXATION

FIELD OF THE INVENTION

The present invention relates to compositions comprising an antioxidant and a nitric oxide (NO) donor or producer. The compositions are useful in the treatment of diseases in which vasoconstriction occurs or is a symptom. Accordingly, methods for treating such diseases are also within the scope of the present invention.

BACKGROUND INFORMATION

The use of nitroglycerin and similar nitrate-type drugs in the treatment of cardiovascular disease is widespread. Nitroglycerin and other clinical nitrates convert into nitric oxide (NO) *in vivo*. Nitric oxide is a key vasodilator for coronary and other vessels such as those in the kidney; NO induced vasodilation can, among other things, relieve the pain of angina caused by insufficient perfusion of coronary vessels of the heart. Nitroglycerin is very fat soluble and can be given, for example, under the tongue, in a mouth spray or on a skin patch; it relieves the pain of angina very quickly because it gets into the blood stream and finds its way to the heart in seconds. When consecutive doses are given, however, the response diminishes quickly. This is because the stretching of the coronary vessels causes the endothelial cell production of superoxide (O_2^-) from oxygen. The superoxide chemically reacts with the nitric oxide (NO) from the nitrate drug and produces peroxynitrite $(OONO)^-$; this reaction diminishes the concentration of nitric oxide available for vasodilation of the coronary vessels. Methods of alleviating this tachyphylaxis have been proposed in which L-arginine is administered in conjunction with nitroglycerin.

For example, U.S. Patent No. 5,543,430 reports a therapeutic mixture comprising L-arginine and a nitrate. Methods of using the mixture to treat a disease condition in a subject by vasodilation (vasorelaxation) are also disclosed. U.S. Patent No. 5,767,160 discloses similar mixtures and methods, using a biological equivalent of arginine instead of L-arginine itself. Use of L-arginine and/or its biological equivalents in these applications, however, has significant drawbacks. L-arginine has side effects, as do other nitrates, that can lead to major toxicity. It causes release of growth hormone which can lead to acromegaly, and is known to produce hyperkalemia in renal failure patients. Significantly, L-arginine and the various nitrates lead to the production of peroxynitrite, a strong oxidizer, which, among other things, causes tissue damage and damage to membrane lipids and DNA of cells. Peroxynitrite has 1000 times the oxidative activity as concentration-equivalent

amounts of hydrogen peroxide, and is therefore a potent oxidizer capable of causing significant damage *in vivo*.

SUMMARY OF THE INVENTION

The present invention relates to compositions comprising one or more nitric oxide (NO) donors and one or more antioxidants. NO donors include L-arginine, arginine derivatives, and nitrates. The present compositions are useful in the generation and maintenance of NO levels *in vivo*. NO is believed to function in the same manner as endothelium-derived relaxing factor (EDRP), which is known to cause vasodilation (vasorelaxation) of the blood vessels. The antioxidants according to the present invention deplete superoxide to minimize if not prevent formation of peroxynitrite, while destroying any peroxynitrite that does form. Thus, NO levels are raised or at least maintained, while peroxynitrite levels are decreased, if not eliminated. In this manner, vasorelaxation can be achieved without the tissue and cellular damage that accompanies peroxynitrite production.

All of the arginine derivatives of the present invention produce more NO per mole of substrate than does L-arginine. Because these derivatives are more efficient precursors for NO production than L-arginine, in combination with supra or mega doses of antioxidant, they produce vasodilation without tachyphylaxisis and without producing the tissue damaged caused by peroxynitrite. This is significant because it allows for the avoidance or minimization of the toxicity associated with the use of L-arginine or nitrates, alone or in combination.

The compositions can further comprise tetrahydrobiopterin (BH_4), and/or hydralazine, both of which inhibit the formation of superoxide. As noted above, superoxide can react with NO to form toxic peroxynitrite.

The present invention further relates to methods of inducing vasorelaxation in a patient comprising administering to the patient one or more NO donors and one or more antioxidants, such as by administration of the compositions of the present invention. The present methods overcome the tachyphylaxis and the production of peroxynitrite that often accompanies multiple administration of NO donors such as nitrates and L-arginine. This is in contrast to various art reported methods, in which NO is generated in sufficient quantity to alleviate tachyphylaxis but superoxide and the peroxynitrite that results are left to cause damage to tissues, membranes and DNA. Various illnesses, such as angina pectoris, in which

vasorelaxation is desired can be treated correctly according to the present methods without short or long-term toxicity.

It is therefore an aspect of the invention to provide compositions that induce vasorelaxation while minimizing the level of peroxynitrite.

Another aspect of the present invention is to provide such compositions for the study and treatment of diseases and disorders in which vasoconstriction or loss of vasodilation is a symptom.

These and other aspects of the invention will be apparent based upon the following description and appended claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the transporter-mediated uptake of FITC-labeled arginine and peptides by alveolar macrophages (AM), determined according to the methods of Example 1, wherein the peptides are actively pumped into the cell.

Figure 2 shows the effects of anti-pepT_1 antibodies on NO production, determined according to the methods of Example 3.

Figure 3 shows the effects of membrane transporter inhibitors on NO production, determined according to the methods of Example 4.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to compositions comprising one or more nitric oxide ("NO") donors. "NO donor(s)" or "NO producer(s)" is used herein to refer to arginine derivatives, L-arginine, and nitrates.

The arginine derivatives used according to the present invention are superior sources of NO when compared to L-arginine itself. L-arginine is a common substrate for two major enzymes--arginase and nitric oxide synthase ("NOS"). Arginase converts L-arginine to L-ornithine and NOS converts L-arginine into citrulline and nitric oxide. There is competition, therefore, between the two enzyme systems for the L-arginine. The present arginine derivatives avoid or at least minimize this competition. As will be discussed below, many of the present arginine derivatives are not suitable substrates for arginase, but are suitable substrates for NOS; thus NO is generated while L-ornithine is not.

"Arginine derivative" is used herein to refer collectively to the following compounds: N^G-hydroxy-L-arginine ("hydroxylated L-arginine"); esters of N^G-hydroxy-L-arginine; amides of N^G-hydroxy-L-arginine; di-, tri- or tetra peptides wherein the first amino

acid at the amino terminal end is L-arginine, N^G-hydroxy-L-arginine, L-homoarginine, N^G-hydroxy-L-homoarginine or esters or amides of any of these amino acids; esters of L-arginine; amides of L-arginine; L-homoarginine, N^G-hydroxy-L-homoarginine or esters or amides of these amino acids; and D-arginine, hydroxylated D-arginine, or esters or amides of these amino acids.

N^G-hydroxy-L-arginine is the direct intermediate in the production of NO. More specifically, L-arginine is metabolized by NOS to an oxidized intermediate known as N^G-hydroxy-L-arginine. N^G-hydroxy-L-arginine reacts with microsomal NOS and inducible NOS to produce NO far better than L-arginine alone. N^G-hydroxy-L-arginine is the key substrate for the various NO synthases, but is not a substrate for arginase, therefore allowing for much greater NO production than L-arginine. The equilibrium constant (Km) for this amino acid is three times greater than L-arginine (6.6 μ Molar compared to 2.3 μ Molar). The maximum velocity at saturation (Vmax) with N-hydroxy-L-arginine as a substrate is 9.9 $\mu M \times min^{-1} \times Mg^{-1}$, as compared to that L-arginine, which is 54 $\mu M \times min^{-1} \times Mg^{-1}$. Thus, less hydroxylated L-arginine is needed to produce NO. N-hydroxy-L-arginine causes vasodilation (vasorelaxation) in a concentration dependent manner similar to L-arginine. The relaxation is inhibited by addition of inhibitors of NO biosynthesis.

The peptides encompassed within the general description of "arginine derivatives" include di-, tri- and tetra- peptides wherein the amino terminal amino acid is L-arginine, hydroxylated L-arginine, L-homoarginine, N-hydroxy-L-homoarginine or ester or amide derivatives of any of these acids. The remaining acid or acids in the peptide are selected from any of the above acids (namely L-arginine or the listed derivatives), or any of the other 19 naturally occurring amino acids or derivatives thereof. Thus, while at least the amino terminal amino acid is L-arginine or a derivative thereof more than one of the amino acids can also be L-arginine or one of its derivatives. "Derivatives of L-arginine" in the present invention refers to hydroxylated L-arginine, L-homoarginine, hydroxylated L-homoarginine, and esters or amides of L-arginine, N^G-hydroxy-L-arginine, L-homoarginine and N-hydroxy-L-homoarginine. Especially preferred peptides include arginine-lysine, arginine-glycine, and arginine-lysine-aspartic acid. L-arginine derivatives can be used in any of the current peptides in place of L-aginine; thus, for ease of reference "arginine-containing peptides" refers collectively to di-, tri- or tetra- peptides having at least one L-arginine or derivative of L-arginine. It is believed that peptides comprising hydroxylated L-arginine are even greater NO substrates than peptides comprising unhydroxylated L-arginine, although the

inventors do not wish to be bound by this. The arginine containing peptides may not produce L-arginine but can react directly with NO synthases. These peptides, as compared to free amino acids, are taken into cells by a specialized transporter known as the proton-coupled oliogopeptide transporter. Selectivity of peptides exceeds selectivity of transport of individual amino acids. Thus, the present arginine-containing peptides are transported into cells without degradation and once internalized in a cell are superior substrates for NO synthases than is L-arginine itself.

L-homoarginine and N-hydroxy-L-homoarginine are also within "arginine derivative" as that term is used herein. These compounds can alone represent the arginine derivative in the present compositions and methods, or can be within the peptides discussed above. L-homoarginine and N-hydroxy-L-homoarginine contain one extra methyl group (-CH_2-) in the carbon chain of arginine. The advantage that the homoisomers of L-arginine have is that they are as good or nearly as good as L-arginine in reacting with NO synthases, but are not substrates of arginase.

Also included within "arginine derivative" as that term is used herein are ester derivatives of the carboxyl end of L-arginine, hydroxylated L-arginine, homoarginine, hydroxylated homoarginine, and the L-arginine or L-arginine derivatives in the arginine-containing peptides. For example, a reaction can occur at the carboxyl end of the amino acid with, for example, acid chlorides or anhydrides to form an ester at the carboxyl end. These derivatizations render the amino acid or peptide "less charged" at the carboxyl end, since the COOH has been esterified. This allows transfer across cell membranes to occur more easily. N-acetyl derivatives, such as L-arginine-acetate, are preferred.

Other derivatives include the amide derivatives of L-arginine, hydroxylated L-arginine, homoarginine, hydroxylated homoarginine, and the L-arginine or L-arginine derivatives in the arginine-containing peptides. Thus, derivitization can also be effected at the amino terminal of the present amino acids.

The final type of "arginine derivative" used in the present invention is D-arginine. As will be appreciated by those skilled in the art, all amino acids occur in either "D" (dextrorotatory) or "L" (levorotatory) form. The natural amino acids in the body are in the "L" form. Although the "D" form is not the preferred substrate for the NO synthases, it still reacts to produce NO in sufficient quantities to elicit a therapeutic benefit. Hydroxylated D-arginine and esters or amide derivatives of D-arginine and hydroxylated D-arginine are within the present invention.

The arginine derivatives of the present invention are preferred substrates for the NO synthases (I, II and III). They produce twice or three times the nitric oxide as the molar equivalent amount of L-arginine. Almost all of the derivatives can be commercially obtained, such as from Bachem in Switzerland or the United States.

L-arginine, as noted above, is a naturally occurring amino acid. It is widely commercially available, such as from Sigma Chemical.

Any substance that produces nitric oxide in solution can be used as the "nitrate" in the present composition, if desired. Preferred are nitrates, including but not limited to nitroglycerin, amyl nitrate, isoamyl nitrate, isorbide dinitrate, isorbide 5-mononitrate and erithrityl tetranitrate; nitroglycerin is most preferred. Nitroglycerin is widely commercially available in a variety of forms including oral, parenteral and dermal. Suitable nitroglycerin is listed in the Drug Handbook 6th Ed. Other suitable compounds include sodium nitroprusside and nitrate esters.

Preferably, the present compositions comprise, as the nitric oxide of donor, a combination of an arginine derivative and a nitrate. The preferable arginine derivatives are hydroxylated L-arginine and di-, tri- or tetra-peptides having L-arginine or hydroxylated L-arginine at the amino terminal. The preferred nitrate is nitroglycerin.

The present compositions also comprise an antioxidant. Any antioxidant that will deplete superoxide levels and/or break down peroxynitrite is within the scope of the present invention. The antioxidant should be in amounts sufficient to minimize peroxynitrite levels; such levels can be readily ascertained using cellular assays known to those skilled in the art. Typically, the antioxidant will be used in what is regarded as a supratherapeutic amount, that is, amounts two times or greater the RDA value for antioxidants having an RDA value. A preferred antioxidant is ascorbic acid, also known as L-ascorbate or Vitamin C, or its derivatives, such as ester C (the calcium salt of Vitamin C), dehydro-L-ascorbate C (an oxidized derivative of Vitamin C), ester C of dehydro-L-ascorbate (an oxidized derivative of ester C) and lipidated derivatives such as ascorbic acid palmitate; these compounds are collectively referred to herein as Vitamin C derivatives or L-ascorbate derivatives. Vitamin C and its derivatives function as antioxidants, converting superoxide molecule to hydrogen peroxide and oxygen. Superoxide, as noted above, combines with NO to form peroxynitrite causing vascular and DNA damage. Supratherapeutic doses of antioxidants, such as Vitamin C or its derivatives, are therefore added to deplete superoxide and serve as an antioxidant against peroxynitrite. This stops the tachyphylaxis and the damage from peroxynitrite which

occurs with use of any of the medical nitrates or L-arginine. It will be understood that other antioxidants, or combinations of antioxidants, are within the scope of the present invention.

The present compositions comprise enough nitric oxide donor or donors to effect a desired level of vasorelaxation in a patient; the composition can therefore have varying concentrations. Administration of the current nitric oxide donor or donors should be in accordance with established practices in the art. For example, Drug Handbook, 6th Edition, provides dosages for various forms of nitroglycerin; one skilled in the art could therefore determine the appropriate amount of nitric oxide donor or donors to administer to a patient to achieve the desired level of NO in that patient. The present compositions can therefore be formulated to contain an appropriate amount of nitric oxide donor or donors. Antioxidants should typically be present so as to result in administration of at least about 150 milligrams per day, and preferably range between 500 milligrams per day and 10,000 milligrams per day.

The composition can further comprise tetrahydrobiopterin (BH_4). BH_4 is a cofactor in the biosynthesis of nitric oxide with NO synthases that converts L-arginine and hydroxyarginine to nitric oxide and citrulline. Tetrahydrobiopterin, if not present in proper amounts, causes the blood vessels to secrete excessive superoxide. If enough tetrahydrobiopterin is added, however, the reaction favors NO production. Typically, a range of doses from between about 10 and 500 milligrams of BH_4 may be added to the current compositions per dose of NO donor.

The present compositions also optionally comprise hydralazine. Hydralazine inhibits the vascular enzyme nicotinamide adenine dinucleotide phosphate (NADP or NADPH) oxidase released upon vasodilation; NADP and NADPH oxidase produce superoxide anions, which in turn combine with NO to produce peroxynitrite. Inhibition of this reaction is therefore beneficial in maintaining levels and minimizing peroxynitrite levels. Hydralazine, if used, should be in an amount sufficient to inhibit NADP oxidase. Typically, this amount will be between about 25 mg and 100 mg, per dose of NO donor.

A preferred composition is one comprising an arginine derivative in conjunction with nitroglycerin as the NO donor, and vitamin C, its derivatives, or combinations thereof as the antioxidant.

The present invention is further directed to a method of treating a patient for an illness comprising administering to the patient an effective amount of NO donor and an antioxidant. The present methods have been found to reduce the tachyphylaxis associated

with introduction of nitroglycerin or other nitrates. More specifically, as discussed above, repeated dosage of nitrates results in reduced response to the drug. The present methods overcome this phenomenon. The NO donor provides a substrate for NO production, providing an agonist of NOS that stimulates conversion of the substrate into NO. The increased NO levels lead to vasorelaxation. The antioxidant minimizes, if not eliminates, peroxynitrite.

"Treating" is intended to encompass both therapeutic and prophylactic treatment of any of the illnesses discussed below; for ease of reference "therapeutic benefit" is therefore used collectively to refer to a benefit that is either therapeutic or prophylactic. A number of therapeutic benefits can be achieved according to the present methods. For example, induction of vasorelaxation leads to blood pressure reduction as well as the pain associated with vasoconstriction. The antioxidant prevents or at least minimizes production of the toxic oxidant peroxynitrite. Reduction of the tachyphylaxis associated with nitrate tolerance is another therapeutic benefit, as is stimulation of NOS and minimization of superoxide production.

The present methods are particularly useful for the treatment of: chronic stable angina; unstable angina; acute myocardial infarction; hibernating myocardium; stunned myocardium; limitation of ventricular remodeling in post myocardial infarction and subsequent risk of congestive heart failure; prophylaxis of recurrent myocardial infarction; prevention of sudden death following myocardial infarction; vasospastic angina; congestive heart failure-systolic; congestive heart failure-diastolic; microvascular angina; silent ischemia; reduction of ventricular ectopic activity; any or all of the states of ischemia myocardium associated with hypertensive heart disease and impaired coronary vasodilator reserve; control of blood pressure in the treatment of hypertensive crisis, perioperative hypertension, preaclampsia or aclampsia; uncomplicated essential hypertension and secondary hypertension; regression of left ventricular hypertrophy; prevention and or regression of epicardial coronary atheriosclerosis; prevention and/or amelioration of free radical mediated reperfusion injury; use in the prevention of myocardial injury during cardioplegic arrest during coronary bypass or other open heart surgery; post transplant cardiomyopathy; renovascular ischemia; cerebrovascular ischemia (transient ischemic attack (TIA) and stroke); and pulmonary hypertension.

"Patient" is used herein to refer to members of the animal kingdom including but not limited to humans. Patients particularly suitable for treatment according to the

present methods include those who need NO, such as those who suffer from an illness in which vasoconstriction is a symptom. Typically, such patients will suffer from high blood pressure, that is a blood pressure of 95-180 mmHg in the systolic range and 55-115 mmHg in the diastolic range.

"Effective amount" is used herein to refer to that amount of the present compositions needed to bring about the desired effect in a patient. Most typically, an effective amount will be the amount that results in raising the NO concentration *in vivo* above basal levels, while minimizing peroxynitrite formation. For example, an effective amount can be that amount needed to induce vasorelaxation while minimizing levels of peroxynitrite; whether a suitable level of vasorelaxation has been achieved can be determined, for example, by monitoring the blood pressure of the patient. An effective amount can also be that amount of the present compounds needed to reduce blood pressure in a patient, or that amount needed to treat tolerance to nitrates or other NOS agonists, while minimizing levels of peroxynitrite. The effective amount will vary depending on various factors including the patient to be treated, the illness being treated, the severity of the illness, the patient's reaction to the treatment, and the like. The determination as to what is an effective amount for each patient is within the skill of those practicing in the art, and can be guided by objective measurements such as blood pressure. An effective amount may be at least about 0.4 mg of NO donor per dose (with 5 doses per day being typical). An effective amount of antioxidant is that amount that will reduce levels of peroxynitrite, typically is at least about 150 mg per day. Any level of vasorelaxation and reduction of peroxynitrite levels is within the scope of the present invention and may be achieved using less than the "typical" amounts listed herein. Similarly, the amount needed to cause the desired level of vasorelaxation in a particular patient may be much higher than the typical effective amount needed to elicit any response.

The term "illness" is used herein to refer to any disease condition that would benefit from the presence of additional NO and/or the induction of vasorelaxation. Examples include, but are not limited to, hypertension, other diseases characterized by high blood pressure, hypertensive heart disease, coronary heart disease including stable, unstable, and microvascular angina, myocardial infarction, hibernating and stunned myocardium, cardiovascular disease including heart failure, stroke and peripheral vascular diseases, renovascular ischemia or hypertension, and congestive heart failure. Any of the conditions listed above in the discussion of therapeutic benefits are also within the scope of the current definition of illness.

As noted above, the present method comprises administering to a patient a NO donor and an antioxidant. It is within the scope of the invention to administer more than one NO donor, and more than one antioxidant, as those terms are described above. Administration of the one or more of each component can be either concurrent or sequential.

Preferably, the components of the present compositions are contained in a pharmaceutically acceptable carrier. "Pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like. The use of such media and agents for pharmaceutically active substances is well known in the art. Use of any of these media or agents is contemplated with the compositions of the present invention, absent compatibility problems with the active compound. Vehicles or carriers standardly used in the pharmaceutical arts for the administration of nitrates, amino acids, and their derivatives and antioxidants can be adapted for use in the present methods by one skilled in the art.

It is especially advantageous to formulate compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the patient to be treated, each unit containing a pre-determined quantity of the active ingredients or "effective amount" calculated to produce the desired therapeutic effect in association with the pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the characteristics of the active ingredients (i.e., the NO donor and antioxidant), the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such active ingredients for the treatment of sensitivity in individuals.

EXAMPLES

The following examples are intended to illustrate the present invention and should not be construed as limiting the invention in any way.

For all the examples fluoroscein isothiocyanate (FITC) labeled lysine (Lys)* and lysine peptides B ala-lys, L-arg-lys, gly-sar-lys were used for cellular uptake studies.

L-arg-lys, L-arg-gly and L-arg-gly-asp were used as arginine containing peptides for transport and utilization studies.

Lysine was used as a competitive inhibitor for CAT-2B (peptide transporter) mediated arginine or lysine uptake. Cephalexin and cephadrine were non-peptide substrates for the peptide transporter (PepT-1).

Antiserums AntiPepT-1, and AntiP455-469 were obtained commercially and used to assess the role of the peptide transport in alveolar macrophage production of nitric oxide (NO).

AM were isolated using non calcium containing physiological buffer. Cell counts were done with a Coulter counter and sizer.

AM were incubated with FITC-substrate for two hours at 37° C. AM were washed and centrifuged three times and finally resuspended in fresh medium. The cell suspension was sonicated for several minutes and fluorescence measured with excitation at 495 nm and emission at 5154 nm.

Aveolar macrophages (AM) were cultured with 10% fetal bovine serum with or without substrate (200 micromolar) and or inhibitor (1 micromolar) at 37° C for two hours. AM conditioned medium was collected and NO measured by accumulated nitrite via the Griess reaction. The concentrations of substrate or inhibitor did not exert toxic effects as assessed by lactate dehydrogenase measurements.

EXAMPLE 1

This example demonstrates the presence of a peptide transporter in alveolar macrophages (AM) for small (2-4) peptide uptake. The example also demonstrates how arginine-containing peptides are utilized by AM in NO production, and the effect of lysophosphatidylchloine (LPC) on the transport and utilization of arginine alone versus arginine-containing peptides by AM. 10 μM of FITC, FITC-labeled lysine, and peptides either with or without 100 μM of lysine and cephradine were incubated with LPS-stimulated AM (106 cells) at 37° C for two hours. The peptides used were arg-lys, B ala-lys, and gly-sar-lys.

Figure 1 demonstrates the presence of a peptide transporter in the plasma membrane of AM. Small peptides, here di- and tri- peptides, were internalized by AM without degradation. The presence of a peptide transporter in the plasma membrane of AM is different from the cationic amino acid transporter that mediates the uptake of arginine and lysine alone.

EXAMPLE 2

This example compares the utilization of L-arginine with arginine-containing peptides by LPS-stimulated AM in production of NO.

200 μM of arginine and argine-containing peptides were incubated with PS-stimulated AM for 24 hours. NO production was determined by measuring the accumulation of nitrite using the Greiss assay.

Utilization of Arginine-Containing Peptide by LPS-Stimulated AM in NO Production

	Nitrite (μM)	
	-LPS	+LPS
AM	1.83 ± 0.27	2.51 ± 0.35
AM + Arg	2.08 ± 0.48	21.52 ± 3.51
AM + Arg-Lys	2.24 ± 1.38	39.27 ± 5.06*
AM + Arg-Gly	2.05 ± 0.36	41.93 ± 3.62*
AM + Arg-Gly-Asp	1.74 ± 1.02	38.42 ± 2.08*

* Significant difference from AM + Arg. $P < 0.05$ $N = 6$

As is seen in the above table, NO production by LPS-stimulated AM when using an L-arginine-containing peptide far exceeded that when using arginine alone. Approximately twice as much NO was produced when using the arginine-containing peptides as a substrate. This demonstrates that arginine-containing peptides are far better substrates for NO production than L-arginine.

EXAMPLE 3

This example demonstrates the effects of anti-pepT1 antibodies on NO production by LPS-stimulated AM. 200 μM of L-arginine and arginine-containing peptides either with or without antiserums were incubated at 37° C with LPS-stimulated AM for 24 hours. The arginine-containing peptides were arg-lys, arg-gly, and arg-gly-asp.

As is seen in Figure 2, the antibody against the peptide transporter blocked the transport of peptides only. It did little against the free amino acid arginine. This confirms that the peptide is transported by the peptide transport system and not the amino acid transport system. Therefore, the arginine transport is different than the arginine peptide transport.

EXAMPLE 4

This example demonstrates the effects of membrane transporter inhibitors on NO production by LPS-stimulated AM. 200 μM of arg-containing peptides either with or without inhibitors (1 μM Lys and cephs, 5 μM LPC) were incubated with LPS-stimulated AM for 24 hours. The arginine-containing peptides were arg-lys, arg-gly, and arg-gly-asp.

As is seen in Figure 3, the amino acid lysine competes with the amino acid arginine for the amino acid transport, but it does not compete with arginine peptide transport. Conversely the antibiotics cephalexin and cephadrine, which use the peptide transporter to enter the cell, compete with the arginine peptides but do not compete with arginine transport, which uses the single amino acid transporter. This shows that the transport of peptides is different than that of single amino acids.

EXAMPLE 5

An 86 year old man with angina pectoris was taking approximately 0.4 milligrams of nitroglycerin five times a day, as prescribed by his doctor. In addition to the nitroglycerin, the man began taking approximately 1,000 milligrams of L-arginine per day and between 3,000 and 5,000 milligrams of L-ascorbic acid per day. Upon treatment with the L-arginine and L-ascorbic acid, the patient experienced a reduction in blood pressure, and a concurrent reduction in the severity and incidents of angina attack.

Whereas particular embodiments of this invention have been described above for purposes of illustration, it will be evident to those skilled in the art that numerous variations of the details of the present invention may be made without departing from the invention as defined in the appended claims.

WHAT IS CLAIMED IS:

1. A composition comprising a nitric oxide donor and an antioxidant.

2. The composition of Claim 1, wherein said nitric oxide donor is one or more members selected from the group consisting of L-arginine, an arginine derivative and a nitrate.

3. The composition of Claim 2, wherein said arginine derivative is N^G-hydroxy-L-arginine.

4. The composition of Claim 2, wherein said arginine derivative is a di, tri- or tetra peptide having an amino terminal member selected from the group consisting of L-arginine and a derivative of L-arginine.

5. The composition of Claim 4, wherein said di- peptide is L-arginine-lysine or hydroxylated L-arginine-lysine.

6. The composition of Claim 4, wherein said di- peptide is L-arginine-glycine or hydroxylated L-arginine-glycine.

7. The composition of Claim 4, wherein said tri- peptide is L-arginine-glycine-aspartic acid or hydroxylated L-arginine-glycine-aspartic acid.

8. The composition of Claim 2, wherein said arginine derivative is an ester derivative of L-arginine, an ester derivative of N^G-hydroxy-L-arginine, an amide derivative of L-arginine, an amide derivative of N^G-hydroxy-L-arginine, L-homoarginine, N^G-hydroxy-L-homoarginine, an ester derivative of L-homoarginine, an ester derivative of N^G-hydroxy-L-homoarginine, an amide derivative of L-homoarginine or an amide derivative of N^G-hydroxy-L-homoarginine.

9. The composition of Claim 2, wherein said arginine derivative is D-arginine.

10. The composition of Claim 2, wherein said nitrate is nitroglycerin.

11. The composition of Claim 2, wherein said nitrate is selected from the group consisting of amyl nitrite, isorbide dinitrate, isorbide 5-mononitrate, erithrityl tetranitrate, sodium nitroprusside, nitrate esters and isoamyl nitrite.

12. The composition of Claim 1, wherein said antioxidant is vitamin C or derivatives thereof.

13. The composition of Claim 12, wherein said nitric oxide donor is a combination of an arginine derivative and nitroglycerin.

14. The composition of Claim 1, further comprising tetrahydrobiopterin.

15. The composition of Claim 1, further comprising hydralazine.

16. A method of treating a patient for an illness comprising administering to said patient an effective amount of:

a) a nitric oxide donor; and

b) an antioxidant.

17. The method of Claim 16, wherein administration of a) and b) is sequential.

18. The method of Claim 16, wherein administration of a) and b) is concurrent.

19. The method of Claim 16, wherein said effective amount of nitric oxide donor is at least about 0.4 mg per dose.

20. The method of Claim 16, wherein said nitric oxide donor is a combination of an arginine derivative and nitroglycerin and said antioxidant is vitamin C or derivatives thereof.

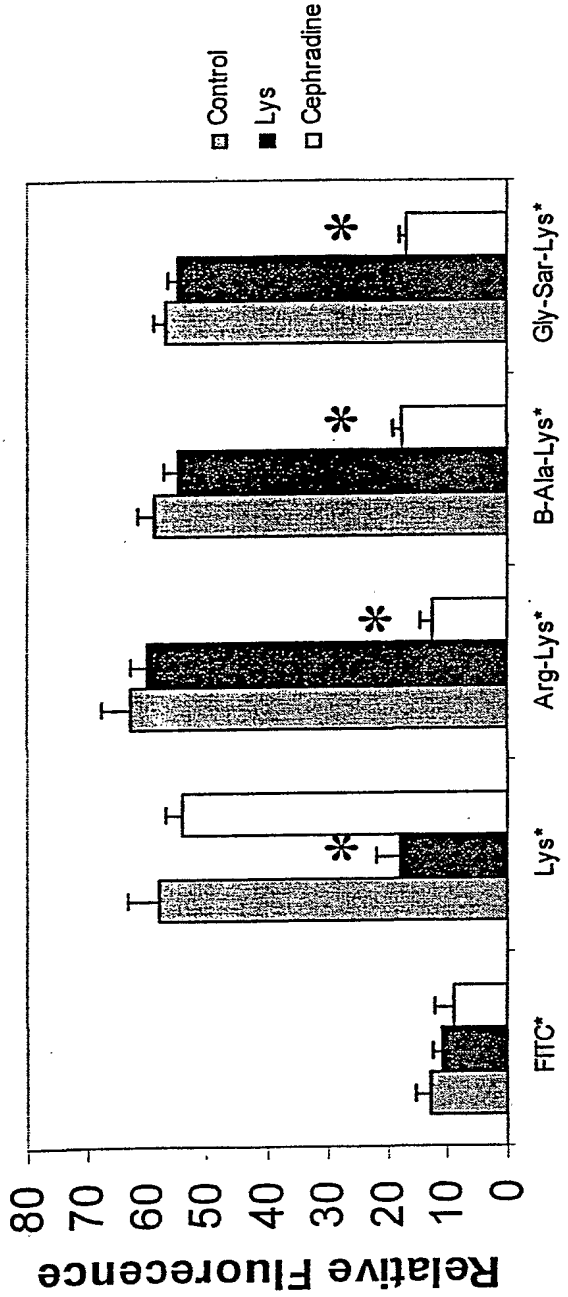
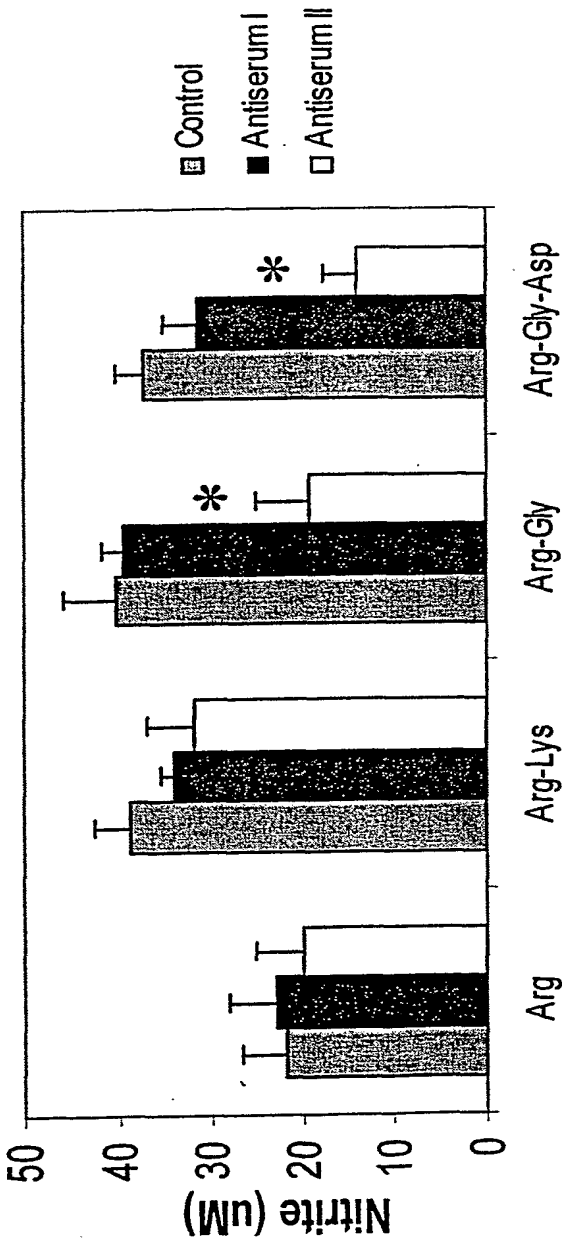

Control
Lys
Cephradine
Relative Fluorescence
0
10
20
30
40
50
60
70
80
FITC*
Lys*
Arg-Lys*
B-Ala-Lys*
Gly-Sar-Lys*
*Significantly lower than control. P < 0.05 N = 6

Figure 1



*Significant different from AM + Arg. $P < 0.05$ $N = 6$

Figure 2

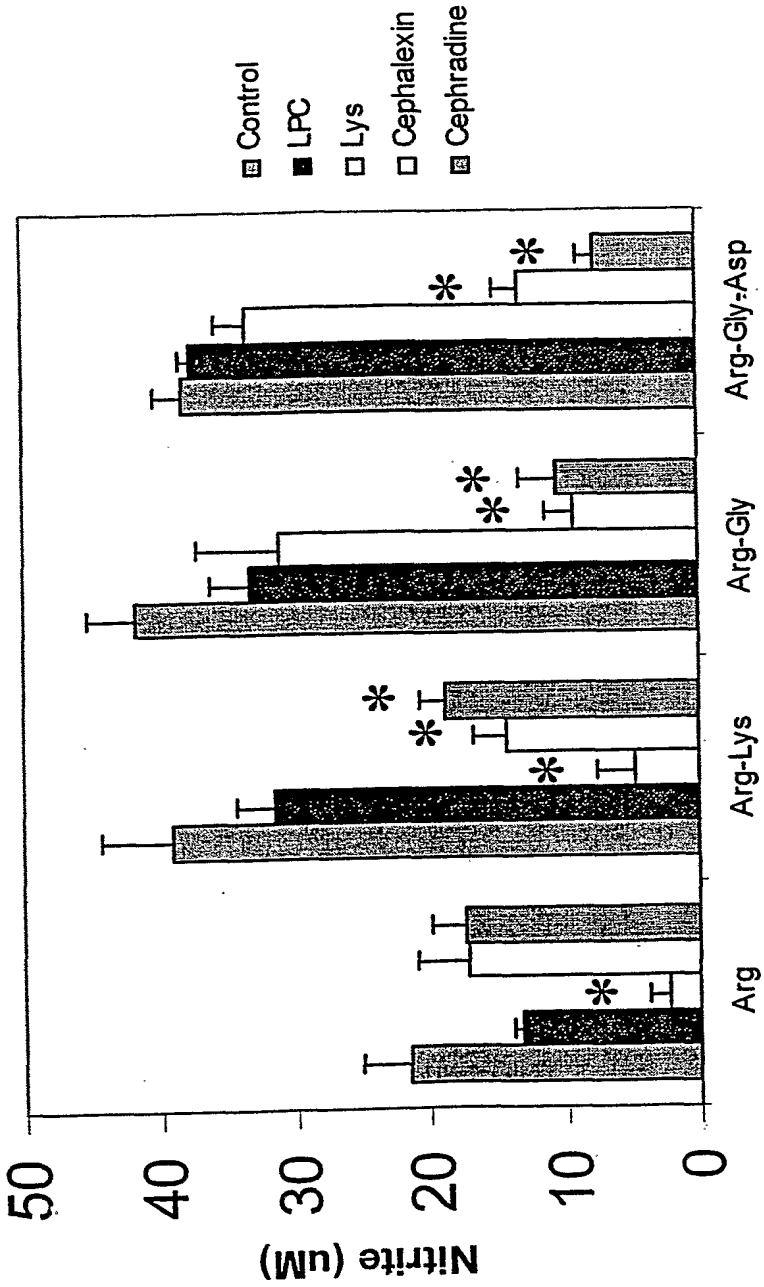

Control
LPC
Lys
Cephalexin
Cephradine
Nitrite (uM)
0
10
20
30
40
50
Arg
Arg-Lys
Arg-Gly
Arg-Gly-Asp
*Significant different from AM + Arg. P < 0.05 N = 6

Figure 3